

09636499-104100

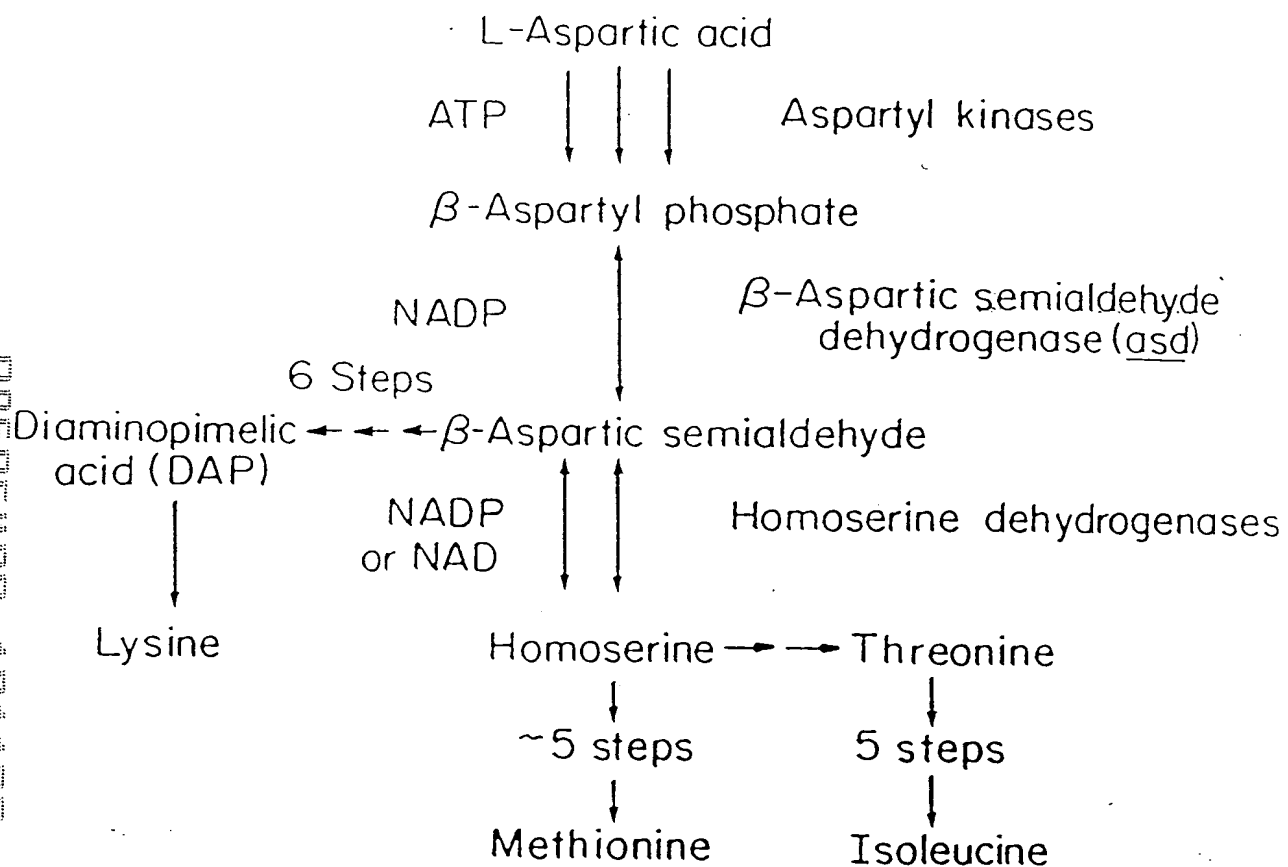


FIGURE 1

FIG. 2

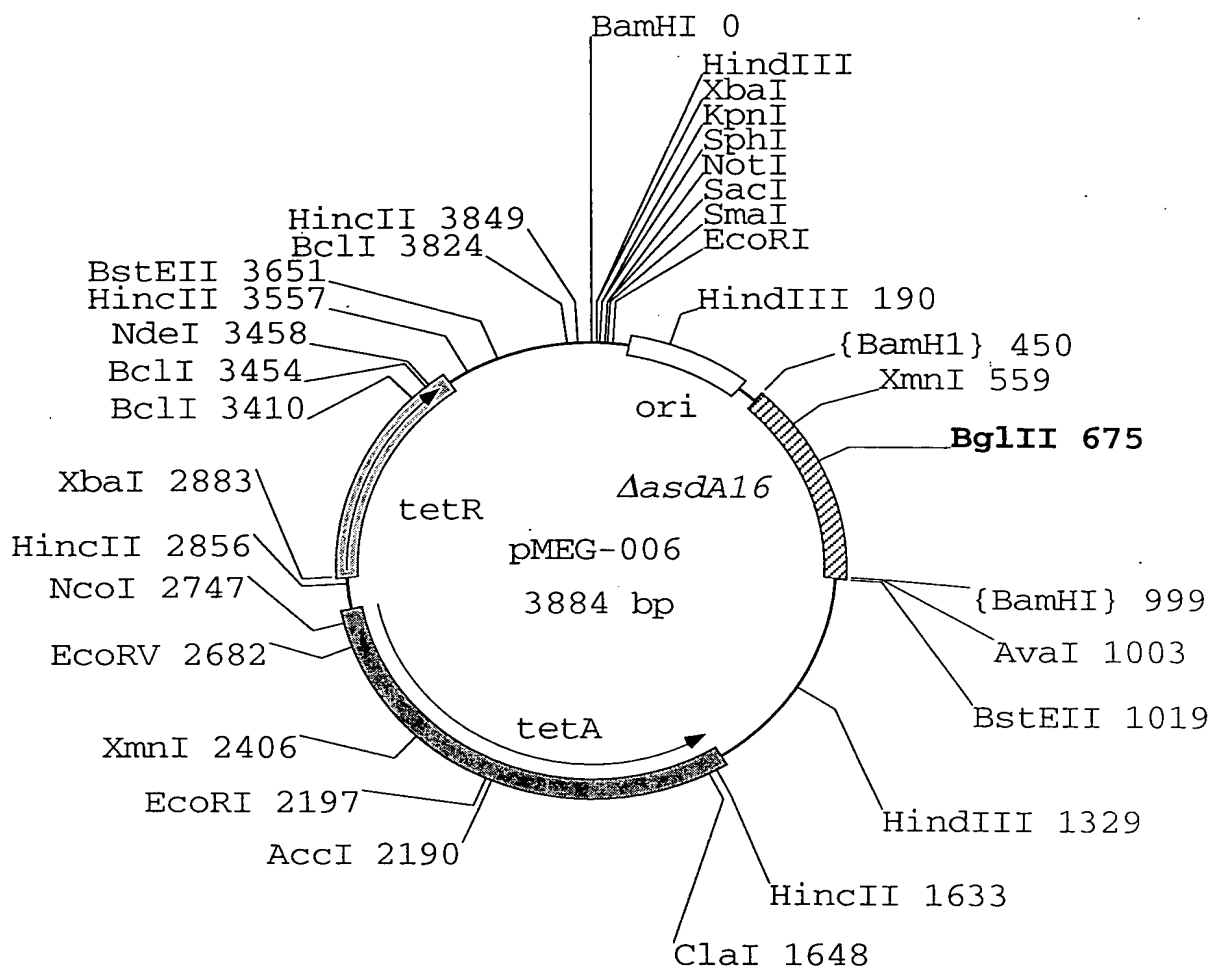
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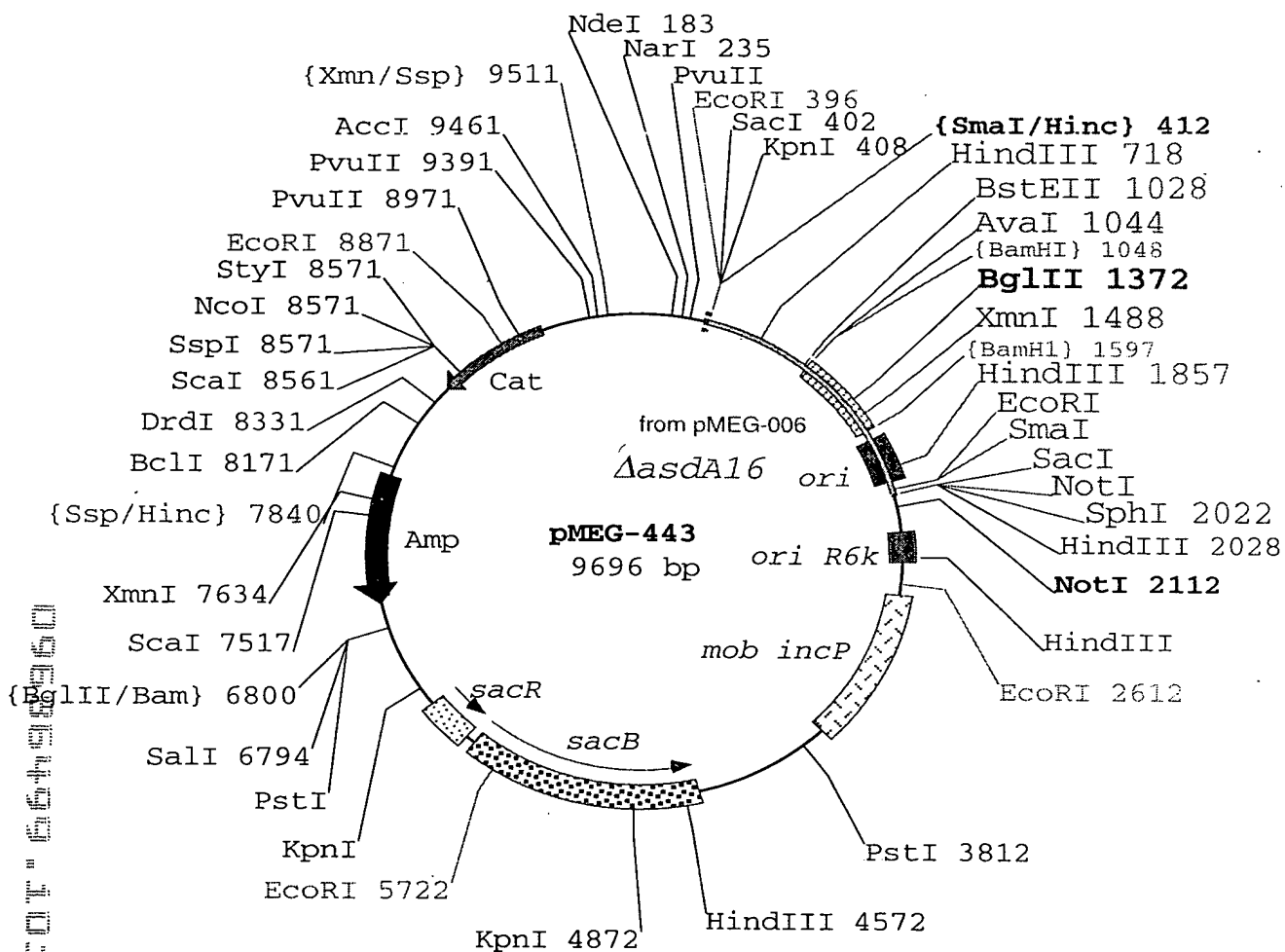
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121 taacgcaaat tccctgataa tcgccactgg actttctgct tgcgcggtaa ggcaggataa
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241 ttgtatgggtg aaggatgcgc cacaggatac tggcgcgcgc acacagcaca tctctttgca
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661 ccaggacgtg attaccgacg gcctgaacaa tggcgtgaag acctttgtgg gcggttaactg
721 taccgttagc ctgatgttga tgcgctggg cggctctctt gcccataatc tcgttgactg
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1441 gggcctgcgt gtggttctgt aggcgggata aggcgcgtca gcgcgccat ccggcgggga
1501 aattttgtgt aaaccagggg tgcacgtca ccctttttt gcgtaataca ggagtaaagc
1561 cagatgtttc atttttatca ggagttaagc agagcattgg ctattcttta agggtagctt
1621 aatccacagg gtattaagcc taacctgaag gtaggacgac gcagatagga tgcacagtgt
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B

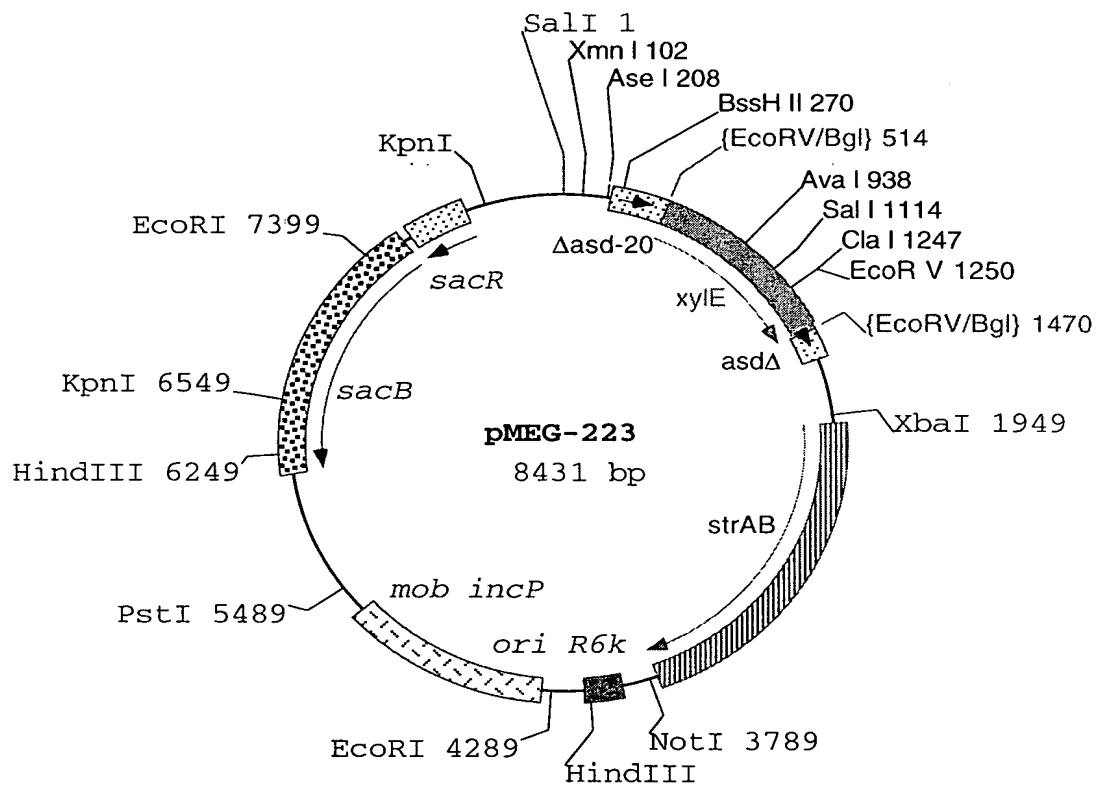
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GHVADELATPSSAILDIERKVTALTRSGELPVDNFGVPLAGSLIPWIDKQLDNGQSREEWKQAETNKI
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$$\{B_0, B_1, B_2, B_3, B_4, B_5, B_6, B_7, B_8, B_9\}$$


0686499.10.100



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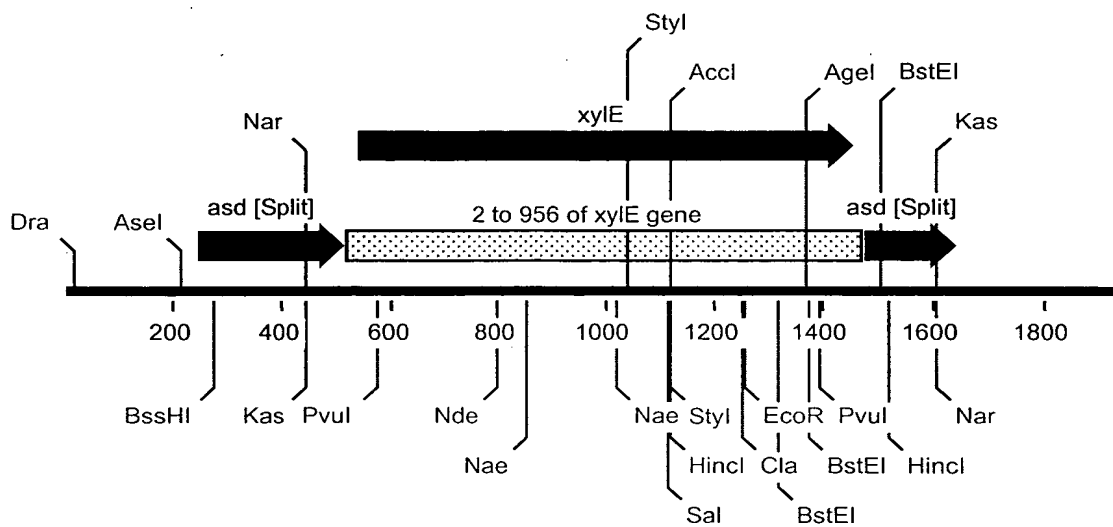


FIG. 6B

DraI

```

      10      20      30      40      50      60
      |
GGATCTTCCCTAAATTTAAATATAAACAACGAATTATCTCCTTAACGTACGTTTTCGTTC

      70      80      90      100      110      120
CATTGGCCCTCAAACCCCTAATTAGGATCAATAAAACAGCGACGGAAATGATTCCTTCC

      130      140      150      160      170      180
TAACGCAAATTCCCTGATAATCGCCACTGGACTTTCTGCTTGCGCGGTAAGGCAGGATAA
  
```

AseI

```

      190      200      210      220      230      240
      |
GTCGCATTACTGATGGCTTCGCTATCATTGATTAATTTCACTTGCGACTTTGGCTGCTTT
  
```

BssHII

```

      250      260      270      280
      |
TTGT ATG GTG AAG GAT GCG CCA CAG GAT ACT GGC GCG CAT ACA CAG
Met Val Lys Asp Ala Pro Gln Asp Thr Gly Ala His Thr Gln
__a__a__a__a__a__a__ASD [SPLIT]__a__a__a__a__a__a__

      290      300      310      320      330
CAC ATC TCT TTG CAG GAA AAA AAC GCT ATG AAA AAT GTT GGT TTT
His Ile Ser Leu Gln Glu Lys Asn Ala Met Lys Asn Val Gly Phe
__a__a__a__a__a__a__ASD [SPLIT]__a__a__a__a__a__a__

      340      350      360      370
ATC GGC TGG CGC GGA ATG GTC GGC TCT GTT CTC ATG CAA CGC ATG
Ile Gly Trp Arg Gly Met Val Gly Ser Val Leu Met Gln Arg Met
__a__a__a__a__a__a__ASD [SPLIT]__a__a__a__a__a__a__

      380      390      400      410      420
GTA GAG GAG CGC GAT TTC GAC GCT ATT CGC CCT GTT TTC TTT TCT
Val Glu Glu Arg Asp Phe Asp Ala Ile Arg Pro Val Phe Phe Ser
__a__a__a__a__a__a__ASD [SPLIT]__a__a__a__a__a__a__
  
```

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NarI
|
KasI
| |

430 440 450 460
ACC TCC CAG TTT GGA CAG GCG GCG CCC ACC TTC GGC GAC ACC TCC
Thr Ser Gln Phe Gly Gln Ala Ala Pro Thr Phe Gly Asp Thr Ser
_a_a_a_a_a_a ASD [SPLIT] _a_a_a_a_a_a
470 480 490 500 510
ACC GGC ACG CTA CAG GAC GCT TTT GAT CTG GAT GCG CTA AAA GCG
Thr Gly Thr Leu Gln Asp Ala Phe Asp Leu Asp Ala Leu Lys Ala
_a_a_a_a_a_a ASD [SPLIT] _a_a_a_a_a_a
520 530 540 550 560
CTC GAT GATCTATGAAGAGGTGACGTC ATG AAC AAA GGT GTA ATG CGA CCG
Leu Asp Met Asn Lys Gly Val Met Arg Pro
a _c_c_c_XYLE_c_c_c_

PvuII
|

570 580 590 600
GGC CAT GTG CAG CTG CGT GTA CTG GAC ATG AGC AAG GCC CTG GAA
Gly His Val Gln Leu Arg Val Leu Asp Met Ser Lys Ala Leu Glu
_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_
610 620 630 640 650
CAC TAC GTC GAG TTG CTG GGC CTG ATC GAG ATG GAC CGT GAC GAC
His Tyr Val Glu Leu Leu Gly Leu Ile Glu Met Asp Arg Asp Asp
_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_
660 670 680 690
CAG GGC CGT GTC TAT CTG AAG GCT TGG ACC GAA GTG GAT AAG TTT
Gln Gly Arg Val Tyr Leu Lys Ala Trp Thr Glu Val Asp Lys Phe
_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_
700 710 720 730 740
TCC CTG GTG CTA CGC GAG GCT GAC GAG CCG GGC ATG GAT TTT ATG
Ser Leu Val Leu Arg Glu Ala Asp Glu Pro Gly Met Asp Phe Met
_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_
750 760 770 780
GGT TTC AAG GTT GTG GAT GAG GAT GCT CTC CGG CAA CTG GAG CGG
Gly Phe Lys Val Val Asp Glu Asp Ala Leu Arg Gln Leu Glu Arg
_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_

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HincII

|

AccI

||

SalIII StyI

| | | |

1110 1120 1130 1140
CAG TTT CTC AGT CTG TCG ACC AAG GCC CAC GAC GTG GCC TTC ATT
Gln Phe Leu Ser Leu Ser Thr Lys Ala His Asp Val Ala Phe Ile
_c_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_c_

1150 1160 1170 1180 1190
CAC CAT CCG GAA AAA GGC CGC CTC CAT CAT GTG TCC TTC CAC CTC
His His Pro Glu Lys Gly Arg Leu His His Val Ser Phe His Leu
_c_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_c_

1200 1210 1220 1230
GAA ACC TGG GAA GAC TTG CTT CGC GCC GCC GAC CTG ATC TCC ATG
Glu Thr Trp Glu Asp Leu Leu Arg Ala Ala Asp Leu Ile Ser Met
_c_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_c_

EcoRV

|

ClaI

|

1240 1250 1260 1270 1280
ACC GAC ACA TCT ATC GAT ATC GGC CCA ACC CGC CAC GGC CTC ACT
Thr Asp Thr Ser Ile Asp Ile Gly Pro Thr Arg His Gly Leu Thr
_c_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_c_

BstEII

|

1290 1300 1310 1320
CAC GGC AAG ACC ATC TAC TTC TTC GAC CCG TCC GGT AAC CGC AAC
His Gly Lys Thr Ile Tyr Phe Phe Asp Pro Ser Gly Asn Arg Asn
_c_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_c_

BstEII

|

AgeI

|

1330 1340 1350 1360 1370
GAA GTG TTC TGC GGG GGA GAT TAC AAC TAC CCG GAC CAC AAA CCG
Glu Val Phe Cys Gly Gly Asp Tyr Asn Tyr Pro Asp His Lys Pro
_c_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_c_

PvuII

1380 1390 1400 1410
GTG ACC TGG ACC ACC GAC CAG CTG GGC AAA GCC TTC TTT TAC CAC
Val Thr Trp Thr Thr Asp Gln Leu Gly Lys Ala Phe Phe Tyr His
___c___c___c___c___c___c___XYLE___c___c___c___c___c___c___

1420 1430 1440 1450
GAC CGC ATT CTC AAC GAA CGA TTC ATG ACC GTG CTG ACC
Asp Arg Ile Leu Asn Glu Arg Phe Met Thr Val Leu Thr
___c___c___c___c___c___c___XYLE___c___c___c___c___c___c___

BstEII

1460 1470 1480 1490 1500
TGATGGTCCGGAGATC ATC ACT ATG CGC GAA TTA ACC CCG GCG GCG GTG
Ile Thr Met Arg Glu Leu Thr Pro Ala Ala Val
___b___b___b___ASD [SPLIT]___b___b___b___b___

HincII

1510 1520 1530 1540 1550
ACC GGC ACG TTG ACT ACG CCG GTT GGT CGT CTG CGT AAG CTG AAC
Thr Gly Thr Leu Thr Thr Pro Val Gly Arg Leu Arg Lys Leu Asn
___b___b___b___b___b___ASD [SPLIT]___b___b___b___b___b___b___

1560 1570 1580 1590
ATG GGC CCA GAG TTC TTG TCG GCG TTT ACC GTA GGC GAC CAG TTG
Met Gly Pro Glu Phe Leu Ser Ala Phe Thr Val Gly Asp Gln Leu
___b___b___b___b___b___ASD [SPLIT]___b___b___b___b___b___b___

NarI

KasI

1600 1610 1620 1630 1640
TTA TGG GGC GCC GCC GAG CCG CTG CGT CGA ATG CTG CGC CAG TTG
Leu Trp Gly Ala Ala Glu Pro Leu Arg Arg Met Leu Arg Gln Leu
___b___b___b___b___b___ASD [SPLIT]___b___b___b___b___b___b___

1650 1660 1670 1680
GCG TAGTGGCTATTGCAGCGCTTATCGGGCCTGCGTGTGG
Ala

1690 1700 1710 1720 1730 1740
TTCTGTAGGCCGGATAAGGCGCGTCAGCGCCGCCATCCGGCGGGGAAATTTGTGTAAAC

Figure 1

Nucleotide sequences of trc promoter/operator and MCS

MCS: *Nco*I *Eco*RI -----*Hind*III

pYA3098, pYA3148, pYA3332, pYA3333, pYA3334,

pYA3336, pYA3339, pYA3340, pYA3341, pYA3342

5' ATTCTGAAATGAGCTGTTGACAATTAATCATCCGGCTC

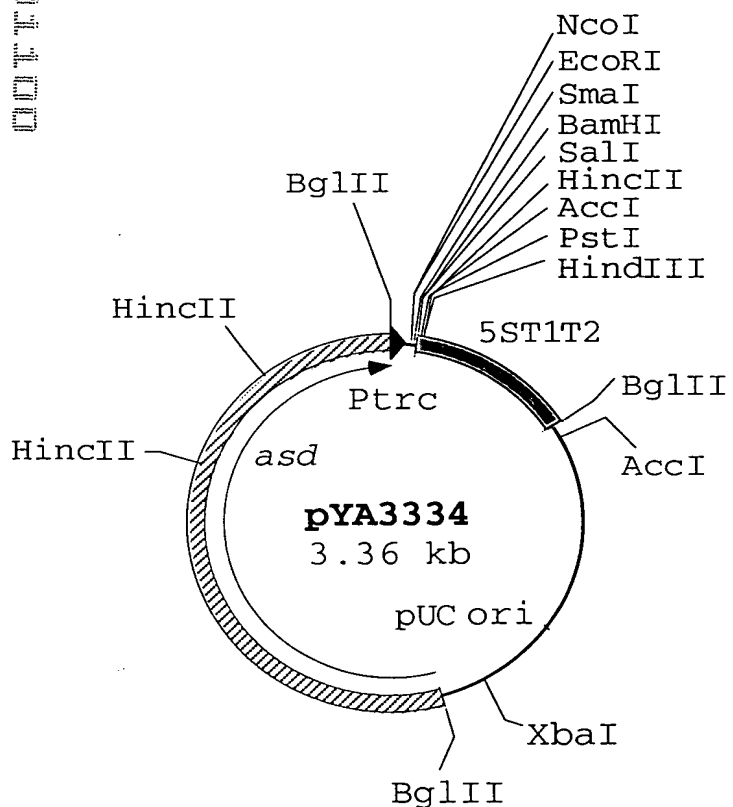
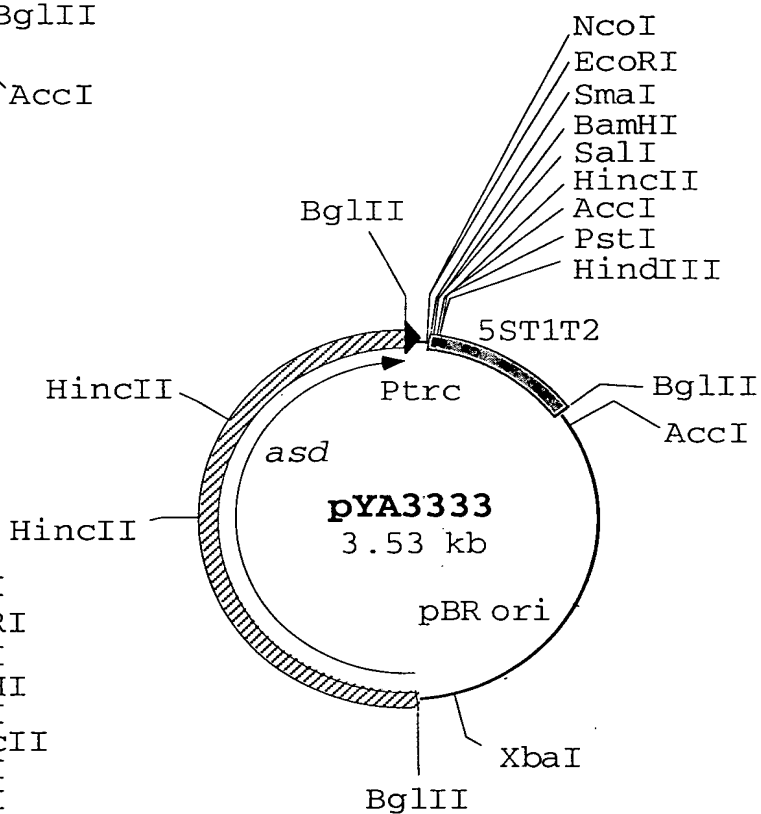
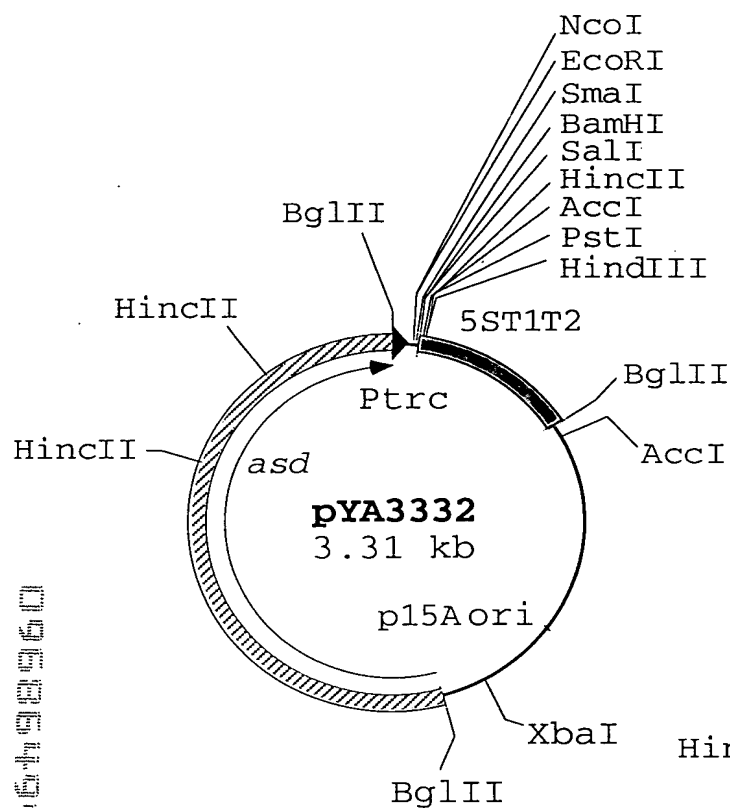
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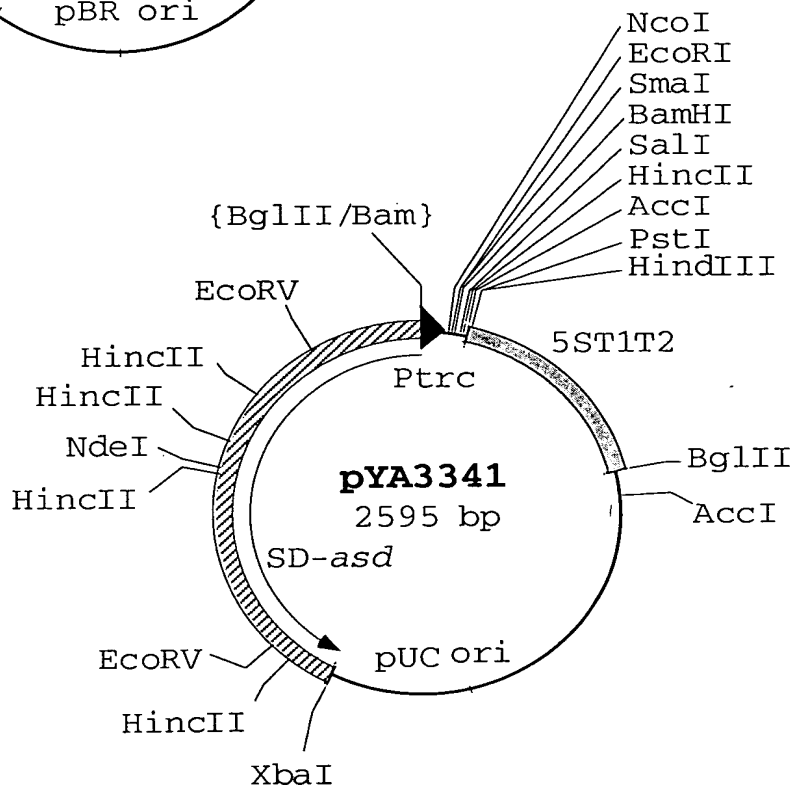
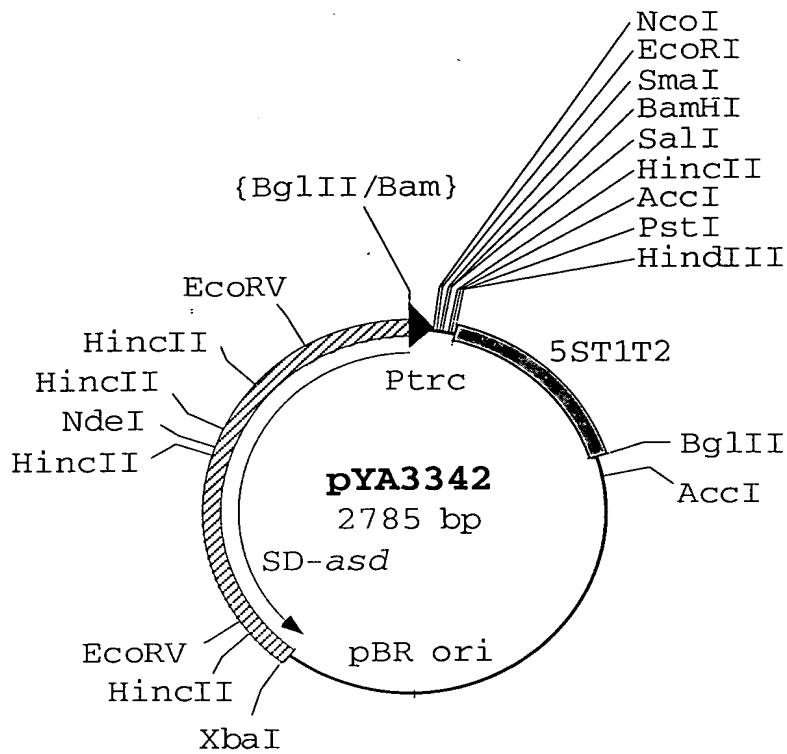
SD NcoI EcoRI SmaI
AGGAAACAGACC ATG GGA ATT CGC AAT TCC CGG GGA
Met Gly Ile Arg Asn Ser Arg Gly

BamHI SalI PstI HindIII
TCC GTC GAC CTG CAG CCA AGC TCC CAA GCT T 3'
Ser Val Asp Leu Gln Pro Ser Ser Gln Ala

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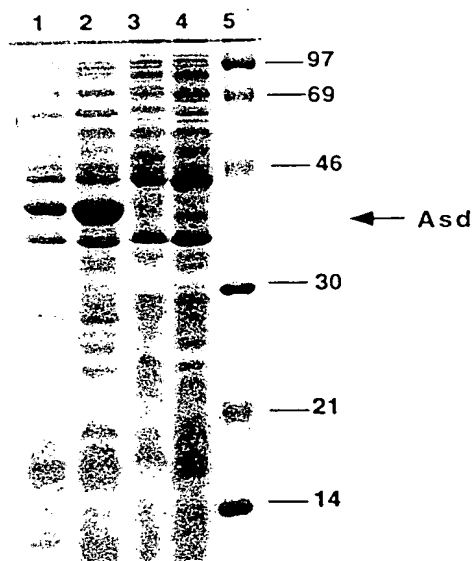
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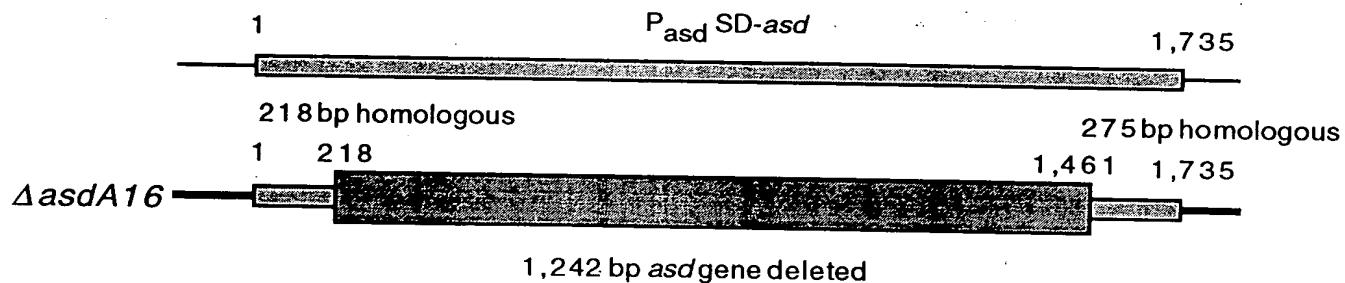
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Level of Asd synthesized in recombinant
S. typhimurium strains with different Asd⁺ plasmids

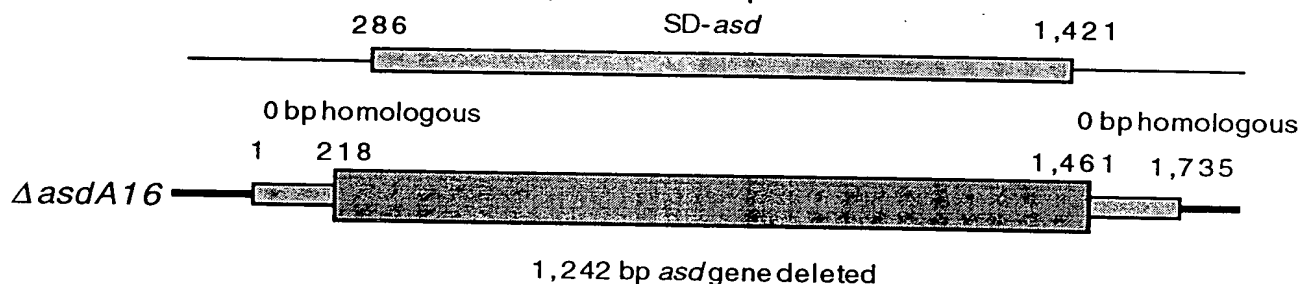


Cell lysates of *S. typhimurium* χ 4550 with pYA3333 (lane 1), pYA3334 (lane 2), pYA3342 (lane 3) and pYA3341 (lane 4). Lane 5 contains molecular weight markers. The arrow indicates Asd protein band.

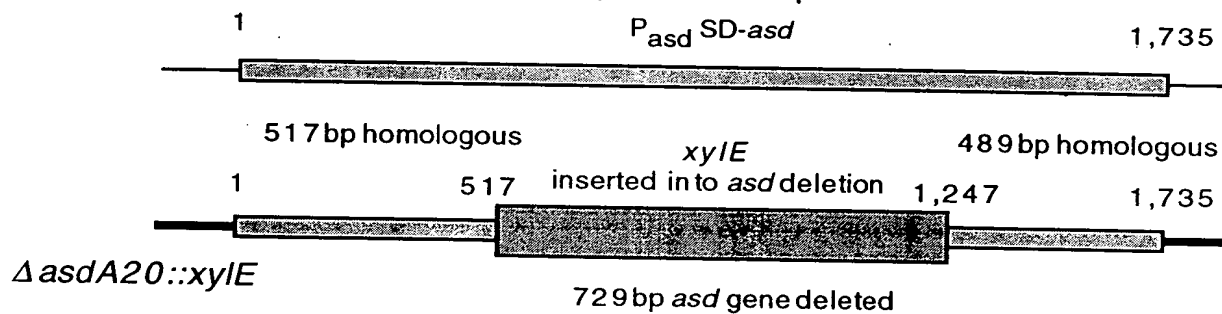
pYA3332, pYA3333 & pYA3334



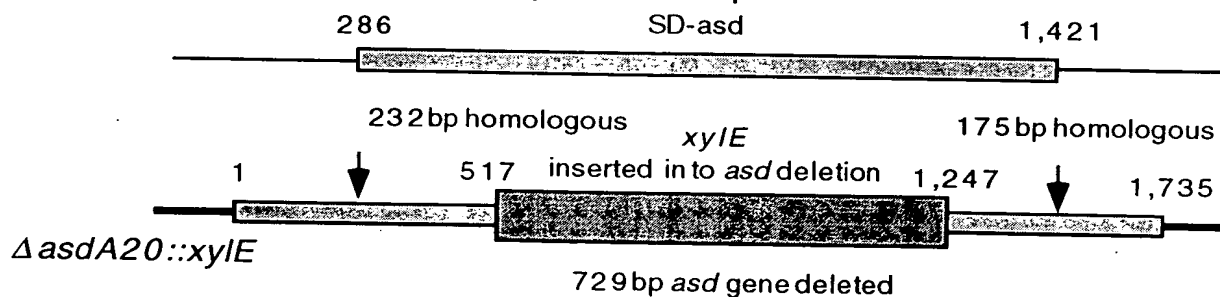
pYA3342 & pYA3341

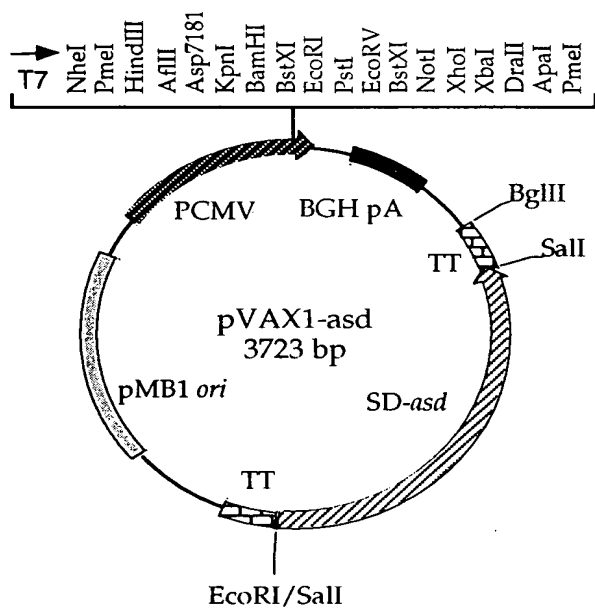


pYA3332, pYA3333 & pYA3334



pYA3342 & pYA3341





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